

09/082,112

L5 ANSWER 1 OF 6 CAPLUS COPYRIGHT 1999 ACS  
AN 1999:236635 CAPLUS  
DN 130:265593  
TI Penicilliosis marneffei and pythiosis Emerging tropical diseases  
AU Kaufman, Leo  
CS Division Bacterial Mycotic Diseases, Centers Disease Control Prevention,  
National Center Infectious Diseases, Atlanta, GA, 30333, USA  
SO Mycopathologia (1998), 143(1), 3-7  
CODEN: MYCPAH; ISSN: 0301-486X  
PB Kluwer Academic Publishers  
DT Journal; General Review  
LA English  
AB A review is given with 28 refs. on penicilliosis marneffei and pythiosis  
insidiosum, emerging infections in subtropical, tropical, and temperate  
areas. Penicilliosis marneffei is endemic in several Southeast Asian  
countries and may be carried to other areas of the world by residents of  
these countries or visitors. Pythiosis occurs in humans and animals who  
frequent the aquatic habitats that harbor **Pythium**  
**insidiosum**. Although early diagnosis is important because of the  
high morbidity or mortality assocd. with these 2 diseases, the diagnosis  
of these infections can be difficult because their clin. and histol.  
features are not pathognomonic. Prompt diagnosis is a prerequisite to  
their appropriate treatment. Lab. testing, involving cultural, histol.,  
and immunol. methods, is necessary to establish an unequivocal diagnosis.  
The clin. presentation, epidemiol., diagnosis and treatment of these

DN 127:204794  
 TI Arachidonic acid and methods for the production and use thereof  
 IN Kyle, David J.  
 PA Martek Corp., USA  
 SO U.S., 11 pp. Cont.-in-part of U.S. Ser. No. 202,878, abandoned.  
 CODEN: USXXAM  
 DT Patent  
 LA English  
 FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 5658767	A	19970819	US 95-367881	19950103
	WO 9621037	A1	19960711	WO 96-US182	19960103
	W:				
	AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI				
	RW:				
	KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN				
	CA 2209513	AA	19960711	CA 96-2209513	19960103
	AU 9648542	A1	19960724	AU 96-48542	19960103
	EP 800584	A1	19971015	EP 96-904435	19960103
	R:				
	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,				
IE	BR 9607179	A	19971111	BR 96-7179	19960103
	CN 1175976	A	19980311	CN 96-192002	19960103
	JP 10512444	T2	19981202	JP 96-521231	19960103
	FI 9702829	A	19970902	FI 97-2829	19970701
	NO 9703085	A	19970903	NO 97-3085	19970702
PRAI	US 91-645454		19910124		
	US 93-35507		19930322		
	US 94-202878		19940228		
	US 95-367881		19950103		
	WO 96-US182		19960103		

AB The present invention relates to processes for the prodn. of arachidonic acid contg. oils, which preferably are substantially free of eicosapentaneic acid. The invention also relates to compns. contg. such oils, in an unmodified form, and to uses of such oils. In a preferred embodiment, *Pythium insidiosum* is cultivated, harvested and the oil is extd., recovered, and used as an additive for infant formula. In an alternative embodiment, *Mortierella alpina* is cultivated, harvested and the oil is extd., recovered, and used as an addi

L5 ANSWER 3 OF 6 CAPLUS COPYRIGHT 1999 ACS  
 AN 1997:107443 CAPLUS  
 DN 126:113184  
 TI Docosahexaenoic acid and/or arachidonic acid for controlling highly  
 unsaturated fatty acid content in various tissues  
 IN Kyle, David J.; Linsert, Henry, Jr.  
 PA Martek Biosciences Corporation, USA; Kyle, David J.; Linsert, Henry, Jr.  
 SO PCT Int. Appl., 79 pp.  
 CODEN: PIXXD2  
 DT Patent  
 LA English  
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9640106	A2	19961219	WO 96-US8649	19960603
	WO 9640106	A3	19970313		
	W:				
	AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG				
	RW:				
	KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA				
	AU 9662521	A1	19961230	AU 96-62521	19960603
	EP 831805	A1	19980401	EP 96-921263	19960603
	R:				
	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
PRAI	US 95-479809		19950607		
	WO 96-US8649		19960603		
AB	A method of treating diseases assocd. with deficiencies in highly unsatd. fatty acids, such as neurol. disorders, comprises administration of a single-cell oil contg. docosahexaenoic acid (DHA), arachidonic acid (ARA) or their combination in an amt. sufficient to elevate the levels of circulating DHA and/or ARA in the person's blood to at least normal levels. A fermn. medium (1 L) contg. glucose and yeast ext. was inoculated with Thraustochytrium aureum and culture was harvested after 9 days to yield .apprx. 4 g dry mass. The DHA content of the lipid in the biom				

L5 ANSWER 4 OF 6 CAPLUS COPYRIGHT 1999 ACS  
 AN 1995:364312 CAPLUS  
 DN 122:123151  
 TI Microbial oils containing arachidonic and docosaheptaenoic acids for  
 treating neurological disorders  
 IN Kyle, David John  
 PA Martek Biosciences Corp., USA  
 SO PCT Int. Appl., 48 pp.  
 CODEN: PIXXD2  
 DT Patent  
 LA English  
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9428913	A1	19941222	WO 94-US6317	19940602
	W: AU, BB, BG, BR, BY, CA, CN, CZ, FI, HU, JP, KP, KR, KZ, LK, LV, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, UZ, VN RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	AU 9469635	A1	19950103	AU 94-69635	19940602
	AU 693450	B2	19980702		
	EP 707487	A1	19960424	EP 94-918217	19940602
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT,				
SE	JP 08511533	T2	19961203	JP 94-501997	19940602
PRAI	US 93-73505		19930609		
	WO 94-US6317		19940602		
AB	A neurol. disorder such as Zellweger's syndrome, Alzheimer's disease, Huntington's disease, schizophrenia, diabetic neuropathy, or neuropathy induced by heavy metal poisoning is treated by administering a microbial oil contg. docosaheptaenoic acid (DHA) or arachidonic acid (ARA) or a combination of DHA and ARA oils in an amt. sufficient to elevate the levels of circulating DHA and/or ARA in the blood to at least normal levels. The oils may be administered in capsules or incorporated into food products (e.g. margarine, salad dressings). Thus, 4500 L of a dextrose-yeast ext. culture of Mortierella alpina was centrifuged and the organisms were dried and extd. with hexane to yield 17 kg crude oil contg. 45% ARA, which was placed in gelatin capsules or processed by conventional vege				

L5 ANSWER 5 OF 6 CAPLUS COPYRIGHT 1999 ACS  
 AN 1992:611313 CAPLUS  
 DN 117:211313  
 TI Microbial oil mixtures containing polyunsaturated long chain fatty acids  
 and their use in infant formulas and parenteral nutrition  
 IN Kyle, David J.  
 PA Martek Corp., USA  
 SO PCT Int. Appl., 38 pp.  
 CODEN: PIXXD2  
 DT Patent  
 LA English  
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9212711	A1	19920806	WO 92-US522	19920122
	W: AT, AU, BB, BG, BR, CA, CH, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MW, NL, NO, PL, RO, RU, SD, SE				
	RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GN, GR, IT, LU, MC, ML, MR, NL, SE, SN, TD, TG				
	CA 2101274	AA	19920725	CA 92-2101274	19920122
	CA 2101274	C	19981215		
	AU 9212392	A1	19920827	AU 92-12392	19920122
	AU 661297	B2	19950720		
	ZA 9200452	A	19921028	ZA 92-452	19920122
	EP 568606	A1	19931110	EP 92-904388	19920122
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, MC, NL, SE				
	BR 9205526	A	19940419	BR 92-5526	19920122
	JP 06505153	T2	19940616	JP 92-504606	19920122
	JP 2731035	B2	19980325		
	IL 100733	A1	19951231	IL 92-100733	19920122
	IL 114253	A1	19970713	IL 92-114253	19920122
	RU 2093996	C1	19971027	RU 93-52410	19920122
	US 5374657	A	19941220	US 92-944739	19920914
	US 5550156	A	19960827	US 94-358474	19941219
PRAI	US 91-645457		19910124		
	WO 92-US522		19920122		
	US 93-944739		19930914		
	IL 95-100733		19950621		
AB	A supplement for infant formula, or for parenteral nutrition, comprises a mixt. of .gtoreq.2 different polyunsatd. long chain fatty acid-contg. microbial oils. Oil contg. 35% docosaheaxaenoic acid (I) isolated from Crypthecodinium cohnii and oil contg. 33% arachidonic acid (II) prepd. from <b>Pythium insidiosum</b> was mixed in a ratio of 1:3 and added to an infant formula. This supplement provides I and II levels equi				

L5 ANSWER 6 OF 6 CAPLUS COPYRIGHT 1999 ACS  
 AN 1992:569911 CAPLUS  
 DN 117:169911  
 TI Arachidonic acid-rich oil manufacture with Pythium or Mortierella as  
 additive for infant formula  
 IN Kyle, David J.  
 PA Martek Corp., USA  
 SO PCT Int. Appl., 27 pp.  
 CODEN: PIXXD2  
 DT Patent  
 LA English  
 FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9213086	A1	19920806	WO 92-US517	19920122
	W: AT, AU, BB, BG, BR, CA, CH, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MW, NL, NO, PL, RO, RU, SD, SE				
	RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GN, GR, IT, LU, MC, ML, MR, NL, SE, SN, TD, TG				
	CA 2101273	AA	19920725	CA 92-2101273	19920122
	AU 9212355	A1	19920827	AU 92-12355	19920122
	AU 661674	B2	19950803		
	ZA 9200454	A	19921028	ZA 92-454	19920122
	EP 568608	A1	19931110	EP 92-904428	19920122
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, MC, NL, SE				
	BR 9205519	A	19940301	BR 92-5519	19920122
	JP 06505384	T2	19940623	JP 92-504604	19920122
	IL 100732	A1	19950629	IL 92-100732	19920122

PRAI US 91-645454 19910124  
 WO 92-US517 19920122

AB The arachidonic acid(I)-rich oil essentially free of eicosapentaenoic acid  
 is manufd. by culturing Pythium or Mortierella and solvent extn. of the biomass. The I-rich oil is also useful for cosmetics and pharmaceuticals.

Aerobic growth of *P. insidiosum* in a medium of tap water, glucose, yeast ext., etc., and extn. of the I-rich oil from the biomass were shown. I-rich oil .apprx.27-28 g was extd. from 100 g dry biomass, and the oil contained arachidonic acid 30-35%.

DN 117:211313  
 TI Microbial oil mixtures containing polyunsaturated long chain fatty acids  
 and their use in infant formulas and parenteral nutrition  
 IN Kyle, David J.  
 PA Martek Corp., USA  
 SO PCT Int. Appl., 38 pp.  
 CODEN: PIXXD2

DT Patent  
 LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9212711	A1	19920806	WO 92-US522	19920122
	W: AT, AU, BB, BG, BR, CA, CH, DE, DK, ES, FI, GB, HU, JP, KP, KR,				
	LK, LU, MG, MW, NL, NO, PL, RO, RU, SD, SE				
	RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GN,				
	GR, IT, LU, MC, ML, MR, NL, SE, SN, TD, TG				
	CA 2101274	AA	19920725	CA 92-2101274	19920122
	CA 2101274	C	19981215		
	AU 9212392	A1	19920827	AU 92-12392	19920122
	AU 661297	B2	19950720		
	ZA 9200452	A	19921028	ZA 92-452	19920122
	EP 568606	A1	19931110	EP 92-904388	19920122
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, MC, NL, SE				
	BR 9205526	A	19940419	BR 92-5526	19920122
	JP 06505153	T2	19940616	JP 92-504606	19920122
	JP 2731035	B2	19980325		
	IL 100733	A1	19951231	IL 92-100733	19920122
	IL 114253	A1	19970713	IL 92-114253	19920122
	RU 2093996	C1	19971027	RU 93-52410	19920122
	US 5374657	A	19941220	US 92-944739	19920914
	US 5550156	A	19960827	US 94-358474	19941219
PRAI	US 91-645457		19910124		
	WO 92-US522		19920122		
	US 93-944739		19930914		
	IL 95-100733		19950621		
AB	A supplement for infant formula, or for parenteral nutrition, comprises a mixt. of .gtoreq.2 different polyunsatd. long chain fatty acid-contg. microbial oils. Oil contg. 35% docosahexaenoic acid (I) isolated from Crypthecodinium cohnii and oil contg. 33% arachidonic acid (II) prepd. from Pythium <i>insidiosum</i> was mixed in a ratio of 1:3 and added to an infant formula. This supplement provides I and II levels equiv. to huma				

TI Arachidonic acid-rich oil manufacture with Pythium or Mortierella as additive for infant formula  
 IN Kyle, David J.  
 PA Martek Corp., USA  
 SO PCT Int. Appl., 27 pp.  
 CODEN: PIXXD2  
 DT Patent  
 LA English  
 FAN.CNT 3

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9213086	A1	19920806	WO 92-US517	19920122
	W: AT, AU, BB, BG, BR, CA, CH, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MW, NL, NO, PL, RO, RU, SD, SE RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GN, GR, IT, LU, MC, ML, MR, NL, SE, SN, TD, TG				
	CA 2101273	AA	19920725	CA 92-2101273	19920122
	AU 9212355	A1	19920827	AU 92-12355	19920122
	AU 661674	B2	19950803		
	ZA 9200454	A	19921028	ZA 92-454	19920122
	EP 568608	A1	19931110	EP 92-904428	19920122
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, MC, NL, SE				
	BR 9205519	A	19940301	BR 92-5519	19920122
	JP 06505384	T2	19940623	JP 92-504604	19920122
	IL 100732	A1	19950629	IL 92-100732	19920122
	JP 11151075	A2	19990608	JP 98-240168	19920122
PRAI	US 91-645454		19910124		
	JP 92-504604		19920122		
	WO 92-US517		19920122		

AB The arachidonic acid(I)-rich oil essentially free of eicosapentaenoic acid  
 is manufd. by culturing Pythium or Mortierella and solvent extn. of the biomass. The I-rich oil is also useful for cosmetics and pharmaceuticals.  
 Aerobic growth of *P. insidiosum* in a medium of tap water, glucose, yeast ext., etc., and extn. of the I-rich oil from the biomass were shown. I-rich oil .apprx.27-28 g was extd. from 100 g dry biomass, and the oil contained arachidonic acid 30-35%.

L18 ANSWER 19 OF 79 CAPLUS COPYRIGHT 1999 ACS

AN 1990:529085 CAPLUS

DN 113:129085

TI Phosphorus-containing glycopolymers of *Clavibacter michiganense* cell walls

AU Varbanets, L. D.; Shashkov, A. S.; Kocharova, N. A.

CS Inst. Microbiol. Virol., Kiev, 252143, USSR

SO Carbohydr. Res. (1990), 204, 157-60

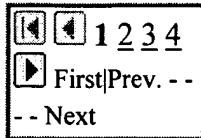
CODEN: CRBRAT; ISSN: 0008-6215

DT Journal

LA English

AB The isolation of cell wall polysaccharides from two strains of *C. michiganense* and the characterization of the teichoic acid component are reported here. The cell wall polysaccharides of two type strains, *C. michiganense* subsp. *michiganense* NCPPB 2979 and *C. michiganense* subsp. *insidiosum* NCPPB 1109, were extd. by mild alk. hydrolysis of the cells. Purifn. of the glycopolymers by ion-exchange chromatog. gave three fractions: the first neutral and comprised of glucans, the next two acidic.





## **Mycoinfo --- Mendoza pg 1**

### ***Pythium insidiosum*: The silent killer of mammals (Leonel Mendoza--02/20/98)**

#### **Introduction and History**

The genus *Pythium* comprises about eighty-five species. *Pythium* species are common pathogens causing disease in plants and fishes. The species of this genus are among the most destructive plant pathogens, inflicting serious economic losses of crops by destroying seed, storage organs, roots, and other plant tissues. *Pythium insidiosum* is the only species reported to cause infections in mammals. The disease caused by this unique microorganism has been termed pythiosis insidiosum and can cause life threatening infections in cats, dogs, cattle, equines, captive polar bears, and humans.

The first published reports of infections caused by *P. insidiosum* were last century in equines with cutaneous granulomas in Florida (USA) and India. Its true etiology, however, was not established. Although several sporadic reports of the disease in equines were made during the beginning of the 20th century, it was not until 1961 that the name *Hyphomyces destruens* was proposed by Bridges and Emmons (*JAVMA* 38:579; 1961) to describe a sterile filamentous microorganism isolated from several cases of cutaneous granulomas in Texas. Later, the binomial *Pythium insidiosum* was proposed by de Cock *et al.*, (*J. Clin. Microbiol.* 25:344;1987). These investigators found that all *P. insidiosum* isolated from humans and animals around the world belonged to the single species *P. insidiosum*. Thus, other binomials used to address this organism became its synonyms.

#### **Taxonomy and Distribution.**

Members of the genus *Pythium* have been described as "aquatic fungi". However, they are not true fungi (Kingdom Fungi), they belong to the Kingdom Chromista, Class Oomycetes, Family Pythiaceae (*Acta Protozool.* 33:1-51; 1994). In culture, *P. insidiosum* develops sparsely septate hyphae similar to those

produced by the Zygomycetes (true fungi). Like other Oomycetes, *P. insidiosum* produces motile zoospores (asexual stage) when exposed to damp conditions. The zoospores are single cells with two lateral flagella that swim to find a new plant host. Once in contact with the host the zoospores lose their flagella and encyst. It is believed that zoospores act as infecting units once in contact with a mammalian host (*J. Mycol. Med.* 6:151; 1996). Under special conditions *P. insidiosum* develops globose oogonia (sexual stage) typical of this species. Pythium species are ubiquitous in soil and aquatic environments. They are worldwide in distribution and have a broad and diverse host range. *Pythium insidiosum* is reported more frequently in tropical and subtropical regions of the world. However, cases in temperate areas of Japan and USA indicate that this organism can be found in cooler environments as well. Well documented cases have been reported in Australia, the Pacific islands, Asia, and the Americas.

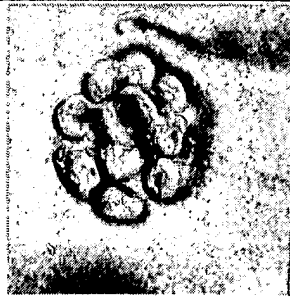


Figure 1. Wet mount of a sporangium of *Pythium insidiosum*.



Figure 2. Scanning electron micrograph of a mature sporangium. Note well developed zoospores and flagella.





In Australia, the disease is restricted to eastern tropical coastal region. In Indonesia, the disease has been reported in islands of Borneo, Java, and Sumatra. In Asia, Japan, Thailand, and India are the countries with more cases of human and animal pythiosis insidiosi. Thailand alone had reported more than forty human cases caused by *P. insidiosum* since 1989. In the Americas, the disease has been reported in Brazil, Colombia, Costa Rica, Haiti, Argentina, and the USA. In the USA pythiosis insidiosi is more frequent in the States along the Gulf of Mexico specially Florida, Louisiana, and Texas where the disease is endemic. Sporadic cases have been recorded in the past ten years in dogs, equines (and even in a captive polar bear) in Georgia, Missouri, North Carolina, South Carolina, Tennessee, and Illinois.

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### Mycoinfo





## **Mycoinfo --- Mendoza pg 2**

### **Epidemiology and Pathogenesis**

Usually the disease is acquired after direct contact with zoospores, or other propagules of *P. insidiosum*, through a skin or mucous membrane injury. It has been well documented that *P. insidiosum* zoospores have an special tropism for open wounds as well as plant tissue (*J. Clin Microbiol.* 31:2967; 1993). This feature allows *P. insidiosum* to direct itself to a new host to complete its life cycle. Once in contact with the host, the zoospores encyst and produce a germ tube that mechanically penetrates the tissue. This happens to mammals entering swampy areas contaminated with this oomycete. In cases of intestinal pythiosis insidiosi in dogs, the infection is usually acquired after ingestion of contaminated water with zoospores. Once in the tissue the host initiates a cell mediated immunoresponse against *P. insidiosum* hyphae. This immunoresponse, however, does not prevent the propagation of the organism into healthy tissue. Most of the damage caused by *P. insidiosum* in tissues is attributed to the release of chemicals from degranulated cells, specially eosinophils and mast cells.

### **Clinical and Pathological Features of Pythiosis insidiosum**

The clinical and pathological changes occurring during *P. insidiosum* infections have been well documented in recent reviews (*J. Med. Mycol.* 6:151; 1996, *Curr. Top. Med. Mycol.* 7:43; 1996, *Vet. Clin. North Am. Equine Pract.* 11:91; 1995). The general clinical and pathological aspects of pythiosis insidiosi in humans and other animals are described in the following sections.

#### **Equine Pythiosis Insidiosi.**

Equine pythiosis insidiosi was first described some time in the last century, but it was not until 1961 that its true nature was determined. The disease in equines has been known under several names such as: equine espundia, Florida horse leeches, leeches, granular dermatitis, hyphomycosis destruens equi, phycomycosis, summer

sores (*llaga brava*, *llaga de verano*), and swamp cancer. Infections caused by *P. insidiosum* in equines are characterized by the formation of cutaneous granulomas. The lesions are more frequently found in body areas first in contact with swampy waters (extremities, thorax, abdomen, and head). The cutaneous granulomas caused by this organism in equines are circular in shape with a characteristic serosanguineous discharge and odor. Lesions seem to be painless, but they are extremely pruritic (itchy). In some cases the lesions are so itchy that the horse bites the affected tissue, complicating the infection. Lesions caused by *P. insidiosum* are also found in bones, intestines, and lungs but these manifestations are more rare. In addition, equine pythiosis insidiosi is clinically similar to a parasitic infection known as equine cutaneous habronemiasis.

In histopathological preparations, *P. insidiosum* produces abundant microabscesses with eosinophils, macrophages and other inflammatory cells. In chronic cases small masses called "kunkers" are observed within the infected granulomas. The coenocytic hyphae (aseptate hyphae) of this oomycete are always found within these masses.

The diagnosis of pythiosis insidiosi in horses is made by culture, histopathology, and/or serology. *Pythium insidiosum* readily grows in Sabouraud agar (+ chloramphenicol) producing aseptate sterile hyphae. In wet preparations containing different ions (specially Ca cations) *P. insidiosum* produces sporangia with zoospores. Histopathologically *P. insidiosum* can be mistaken with hyphae of the Zygomycetes (fungi). However, an immunoperoxidase assay is available to specifically detect the organism in the infected tissues. Two serological tests have been used in the past ten years: they are; immunodiffusion (ID) and Pythium-ELISA. Both are specific but the ELISA is more sensitive (*J. Clin. Microbiol.* 23:813; 1986, *Clin. Diag. Lab. Immunol.* 4:715; 1997). In addition, western blot analysis shows to be of value in the investigation *P. insidiosum* immunogens during infection (*J. Clin. Microbiol.* 30:2980; 1992).

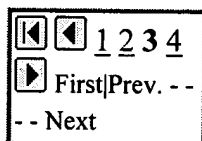
The traditional treatment of equine pythiosis insidiosi is radical surgery of the cutaneous granulomas. Recently, a neodymium-yttrium-aluminum-garnet (YAG) laser for treatment of pythiosis granulomas in two horses was successfully applied (*JAVMA* 211:464; 1997). Amphotericin B and iodides have been used in the

chemotherapy of equine pythiosis. However, both drugs are toxic and the results obtained over the past twenty years are controversial. Immunotherapy using a vaccine proved to be very successful to cure the disease in equines. This curative vaccine contains proteins of *P. insidiosum* and it is recommended in horses with active pythiosis insidiosi (*Mycopathologia* 119:89; 1992, *The Compendium* 15:491; 1993). The vaccine has been successful in more than 300 cases. Presently, its prophylactic (protective) features are under investigation. If the infection is not treated in the initial stages it is 100% fatal.

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### Mycoinfo





## **Mycoinfo --- Mendoza pg 3**

### **Dog Pythiosis Insidios**

Infections caused by *P. insidiosum* in dogs have been reported in endemic areas of the United States and in other countries. The disease is characterized by developments of cutaneous and intestinal granulomas. As in equines, the disease in dogs is acquired through skin or mucous membrane injury. Dogs residing in the country side and those visiting endemic areas are prone to infection.

Two clinical signs are often observed in dogs infected with this oomycete: cutaneous lesions, and gastrointestinal granulomatous lesions. Cutaneous pythiosis in dogs is acquired through traumatic implantation of *P. insidiosum* into the skin, whereas gastrointestinal pythiosis insidios is acquired through ingestion of water contaminated with zoospores. The skin lesions are usually present on the legs, face and tail. Cutaneous lesions are itchy with sinus tracts. Ulceration of the original skin granulomas are frequent. Intestinal pythiosis insidios in dogs is characterized by severe weight loss, vomiting and diarrhea. The granulomatous gastrointestinal masses caused by *P. insidiosum* mimic those observed in neoplastic diseases, thus differential diagnosis is crucial. If not treated the disease is lethal.

As in equine pythiosis insidios the diagnosis is based on cultural, histopathological, and serological techniques. The most important methods for diagnosis are the immunoperoxidase and the immunodiffusion tests. Culture is also important, but just a few laboratories have the expertise required to identify this pathogen.

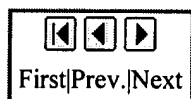
If the infection is not detected early the infected dogs usually die. According to recent data the number of dogs having this disease has enormously increased in the past five years. This is due to increasing awareness of *P. insidiosum* in endemic areas and the dissemination of knowledge about its clinical, diagnostic, and epidemiological features. Treatment in most cases is not successful due to the chronicity of the lesions.

## Pythiosis Insidiososi in Other Animals

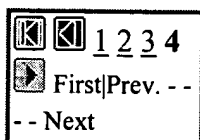
In addition, pythiosis insidiososi has been reported in several cats, cattle, and in a captive polar bear. In these cases the infection was localized in the subcutaneous tissues. The diagnosis and treatment is similar to those used in dogs and equines.

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### Mycoinfo







## **Mycoinfo --- Mendoza pg 4**

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### **Pythiosis Insidiosum in Humans**

Human pythiosis insidiosum is characterized by the formation of subcutaneous lesions and the invasion of the main arteries. If not treated the infection is fatal. The majority of the cases have been diagnosed in Thailand. The disease has been also reported in Australia, Haiti, India, and the USA. The organism is acquired through traumatic implantation and remains localized or spreads to infect other tissues, especially arteries. The diagnosis of the disease in humans is based in culture, serology, and histopathology. In all cases, hyphae of this oomycete are present in the infected tissues. Serological test such as ID and ELISA have proved to be of value for its early diagnosis.

Treatment of human subcutaneous pythiosis insidiosum on limbs, in which the arteries have been involved, consists of the amputation in the affected extremity. Iodides and other drugs have been used with questionable results. More recently the vaccine used to treat equine pythiosis, was successfully used in at least two cases of human pythiosis. The vaccine is being investigated for its possible use in new cases of human and animal pythiosis insidiosum.

Infections caused by this microorganism should no longer be considered rare in equines and companion animals. Dog and cat owners inhabiting endemic areas should consult their DVM practitioners when their pets present ulcerative cutaneous lesions or severe weight loss, vomiting, and diarrhea, for possible pythiosis insidiosum infection. I call this organism "the silent killer of mammals" because its true etiology was only recently being considered as a differential diagnosis with other similar clinical entities. The more we know about *P. insidiosum* infections and treatment the better prepared we are in preventing the disease and saving lives.

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Department of Microbiology  
Michigan State University, USA  
<mailto:mendoza9@pilot.msu.edu>

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L2 ANSWER 19 OF 20 MEDLINE  
 AN 88087823 MEDLINE  
 DN 88087823  
 TI Antigenic relationship between the animal and **human** pathogen  
 Pythium insidiosum and nonpathogenic Pythium species.  
 AU Mendoza L; Kaufman L; Standard P  
 CS Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa  
 Rica.  
 SO JOURNAL OF CLINICAL MICROBIOLOGY, (1987 Nov) 25 (11) 2159-62.  
 Journal code: HSH. ISSN: 0095-1137.  
 CY United States  
 DT Journal; Article; (JOURNAL ARTICLE)  
 LA English  
 FS Priority Journals  
 EM 198804  
 AB Identification of the newly named pathogenic oomycete Pythium insidiosum  
 and its differentiation from other Pythium species by morphologic  
 criteria  
 alone can be difficult and time-consuming. Antigenic analysis by  
 fluorescent-antibody and immunodiffusion precipitin techniques  
 demonstrated that the P. insidiosum isolates that cause **pythiosis**  
 in dogs, horses, and **humans** are identical and that they were  
 distinguishable from other Pythium species by these means. The  
 immunologic  
 data agreed with the morphologic data. This indicated that the animal and  
**human** isolates belonged to a single species, P. insidiosum.  
 Fluorescent-antibody and immunodiffusion reagents were developed for the  
 specif

L6 ANSWER 7 OF 8 MEDLINE  
 AN 79061586 MEDLINE  
 DN 79061586  
 TI Prevention of surface bacterial contamination of donor corneas.  
 AU Goldman K N; Centifanto Y; Kaufman H E; Slappeg T E  
 SO ARCHIVES OF OPHTHALMOLOGY, (1978 Dec) 96 (12) 2277-80.  
 Journal code: 830. ISSN: 0003-9950.  
 CY United States  
 DT Journal; Article; (JOURNAL ARTICLE)  
 LA English  
 FS Abridged Index Medicus Journals; Priority Journals  
 EM 197903  
 AB A simple method has been developed to reduce contamination in postmortem donor human eyes in anticipation of corneal transplantation. In vivo investigation of albino rabbits demonstrates that vigorous saline solution irrigation is extremely effective in decreasing the surface bacterial counts of the postmortem eye. In vitro and in vivo studies show that Neosporin kills bacteria at room temperature and further show that a tenfold increase in the thimerosal concentration of the Neosporin will kill fungus. Postmortem eyes contaminated by pathogenic organisms can be effectively cleaned by a combination of saline solution irrigation and the new Neosporin-thimerosal solution. No substantial damage of the donor tissue was noted by scanning electron microscopy. Human eyes cultured before this procedure were all contaminated, but after cleansing and immersion, no bacterial or fungal growth occurred.

L2 ANSWER 8 OF 20 MEDLINE  
AN 93375115 MEDLINE  
DN 93375115  
TI **Human** subcutaneous **pythiosis**.  
AU Triscott J A; Weedon D; Cabana E  
CS Department of Anatomical Pathology, Royal Brisbane Hospital, Australia..  
SO JOURNAL OF CUTANEOUS PATHOLOGY, (1993 Jun) 20 (3) 267-71.  
Journal code: HWM. ISSN: 0303-6987.  
CY Denmark  
DT Journal; Article; (JOURNAL ARTICLE)  
LA English  
FS Priority Journals  
EM 199312  
AB Two cases of subcutaneous infection caused by the primitive aquatic  
hyphal  
organism *Pythium* are described. *Pythium* is an important pathogen of  
horses  
in the U.S.A. and Australia. Cases of **human** subcutaneous  
**pythiosis** have been cited in the literature, but clinical and  
histopathological features have not been described previously. Both cases  
occurred in young immunocompetent males in the periorbital region and  
showed rapid growth, clinically mimicking a tumor and requiring operative  
biopsy. In both cases there was a history of exposure to either swampy  
water or horses. The tissue reaction was distinctive, closely resembling  
that seen in equine **pythiosis**, comprising well-defined granular  
eosinophilic islands bordered by macrophages, multinucleate giant cells,  
fibrosis and numerous eosinophils. Hyphae were well demonstrated with the  
Grocott stain but only poorly with the PAS method. Identity of the  
organisms was confirmed with an immunoperoxidase technique employing a  
polyclonal antiserum to *Pythium*. Both patients responded well to  
amphot

L2 ANSWER 7 OF 20 MEDLINE  
 AN 94086797 MEDLINE  
 DN 94086797  
 TI Life cycle of the **human** and animal oomycete pathogen *Pythium*  
*insidiosum* [published erratum appears in J Clin Microbiol 1994  
 Jan;32(1):276].  
 AU Mendoza L; Hernandez F; Ajello L  
 CS Division of Biological Sciences, University of Texas at Austin  
 78712-1095..  
 SO JOURNAL OF CLINICAL MICROBIOLOGY, (1993 Nov) 31 (11) 2967-73.  
 Journal code: HSH. ISSN: 0095-1137.  
 CY United States  
 DT Journal; Article; (JOURNAL ARTICLE)  
 LA English  
 FS Priority Journals  
 EM 199403  
 AB *Pythium insidiosum*, the etiologic agent of **pythiosis** *insidiosii*,  
 causes life-threatening infections in **humans** and animals.  
 Previous studies of the epidemiology of this disease hypothesized about  
 the possible life cycle of this oomycete. Details, however, were not  
 provided on the steps required to cause infection. We investigated the  
 life cycle of *P. insidiosum* by inoculating pieces of equine skin and  
 plant  
 leaves and then studying the ensuing events with a scanning electron  
 microscope. Our observations revealed that zoospores had a strong tropism  
 for skin tissue, horse and **human** hair, and water lily and grass  
 leaves and a weak attraction to a variety of other leaves. Encysted  
 zoospores were observed on the favored leaves and skin. There they  
 produced germ tubes and later abundant hyphal filaments that penetrated  
 leaf tissues. Young sporangia had compact, thick walls. The sporangial  
 wall was reduced to a fragile membrane when the sporangia had produced  
 well-differentiated biflagellate zoospores. The encysted zoospores  
 secreted an amorphous material that permitted the zoospores to adhere to  
 skin and plant tissues. On the basis of these findings, a model to  
 explain

L2 ANSWER 6 OF 20 MEDLINE  
 AN 95023507 MEDLINE  
 DN 95023507  
 TI **Human pythiosis** in Thailand.  
 AU Imwidthaya P  
 CS Department of Microbiology, Siriraj Hospital, Mahidol University,  
 Bangkok,  
 Thailand..  
 SO POSTGRADUATE MEDICAL JOURNAL, (1994 Aug) 70 (826) 558-60. Ref: 18  
 Journal code: PFX. ISSN: 0032-5473.  
 CY ENGLAND: United Kingdom  
 DT Journal; Article; (JOURNAL ARTICLE)  
 General Review; (REVIEW)  
 (REVIEW, TUTORIAL)  
 LA English  
 FS Priority Journals  
 EM 199501  
 AB Pythium insidiosum is a protocista and causes diseases in plants and animals. In Thailand it can cause a unique **human** infection of three types. The first type is a subcutaneous lesion in thalassaemic patients, with the pathological findings of a granulomatous reaction, diffuse infiltration, and oedema of the vessel walls. The patients responded to a saturated solution of potassium iodide. The second type is chronic inflammation and occlusion of blood vessels mainly in the lower extremities which results in gangrene or aneurysm formation. This type of infection is only found in thalassaemic patients and leads to amputation of the affected extremities or resection of the involved arteries. The third type is keratitis. This type of infection may or may not be associated with thalassaemia. The clinical signs and symptoms do not differentiate it from other types of mycotic keratitis. The patients end up with keratoplasty, evisceration or enucleation. Thailand is an agricultural country, and there are plenty of swampy areas and several plants to support the life cycle of Pythium. Moreover, many people suffer from thalassaemia, and there is no drug available for Pythium.  
**Pythi**

DN 98043951  
 TI Serodiagnosis of **human** and animal **pythiosis** using an  
 enzyme-linked immunosorbent assay.  
 AU Mendoza L; Kaufman L; Mandy W; Glass R  
 CS Medical Technology Program, Michigan State University, East Lansing  
 48824-1031, USA.. mendoza9@pilot.msu.edu  
 SO CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (1997 Nov) 4 (6) 715-8.  
 Journal code: CB7. ISSN: 1071-412X.  
 CY United States  
 DT Journal; Article; (JOURNAL ARTICLE)  
 LA English  
 FS Priority Journals  
 EM 199803  
 EW 19980302  
 AB Conventional serodiagnosis of *Pythium insidiosum* infections involves the  
 use of the immunodiffusion (ID) test. This test specifically diagnoses  
**human** and animal **pythiosis**. The test, however, has  
 limited sensitivity and does not detect some culturally proven cases of  
 the disease. Because of the increased recognition of **pythiosis**  
 among **humans** and animals, we developed and evaluated an  
 enzyme-linked immunosorbent assay (ELISA) using a soluble antigen from  
 broken hyphae of *P. insidiosum*. Studies were carried out with sera from  
 five **humans** and eight animals with culturally and/or  
 histologically proven **pythiosis**. Some of these sera were  
 negative in the ID test for **pythiosis**. Heterologous case sera  
 from thirteen **humans** and two horses, plus 5 sera from healthy  
**humans** and 17 from healthy animals, were tested. Of the  
**pythiosis** case sera tested, the ID test detected only 8 of 13  
 (61.5%), whereas the ELISA detected all of them (100%). The ID and ELISA  
 tests were entirely specific and gave negative results or low titers  
 respectively, with sera from **humans** and animals with  
 heterologous fungal infections or with no apparent illness. No  
 correlation  
 was found between the height of the ELISA titers and negative or positive  
 sera in the ID test. Our results indicate that the ELISA is a reliable  
 serodiagnostic test for **pythiosis**. It is as specific as the ID  
 test b

L2 ANSWER 3 OF 20 MEDLINE  
 AN 1998164809 MEDLINE  
 DN 98164809  
 TI **Human pythiosis.**  
 AU Thianprasit M; Chaiprasert A; Imwidthaya P  
 CS Department of Dermatology, Siriraj Hospital, Mahidol University, Bangkok, Thailand.  
 SO CURRENT TOPICS IN MEDICAL MYCOLOGY, (1996 Dec) 7 (1) 43-54. Ref: 72  
 Journal code: CTM. ISSN: 0177-4204.  
 CY Spain  
 DT Journal; Article; (JOURNAL ARTICLE)  
 General Review; (REVIEW)  
 (REVIEW OF REPORTED CASES)  
 (REVIEW, TUTORIAL)  
 LA English  
 FS Priority Journals  
 EM 199805  
 EW 19980504

AB **Pythiosis** is a cosmopolitan granulomatous disease caused by an aquatic fungus *Pythium insidiosum* which usually occurs in horses, cattle, dogs, cats or fishes. There have been 28 cases of **human pythiosis** published in the literature. Twenty three patients have been reported from all over Thailand. **Human pythiosis** presents in one of three clinical forms: cutaneous or subcutaneous, systemic or vascular and ophthalmic (e.g., corneal ulcer or keratitis). Systemic antibiotics or antimycotics are not effective in the treatment

of  
 this infection. A saturated solution of KI gives a beneficial result only in the subcutaneous form. Surgical removal of the source of infection is the m



# **Test Your Knowledge**

**Marisa Pasekoff '00, Recorder SVECCS  
University of Florida**

## **"Sabrina"**

Signalment: 1 year old, Female, German Shepherd

**History:** Sabrina presented as an emergency to the dermatology department (can you believe that one - a derm emergency!) on 1/1/98 with a lesion on her rump that had been there since 10/98. There had been a history of draining tracts and the lesion seemed to have progressed cranially to the lumbosacral area. She had been treated with a first generation cephalosporin for two months and enrofloxacin for ten days, neither of which helped. The referring veterinarian amputated the tail in December of 1998 and sent it in for histopathology. The pathologist said the tissue sample did not reveal an infectious cause but it could not be ruled out. Sabrina was current on her vaccinations (DA2PP and rabies) and she was not due for her boosters until April. She was given Heartgard 30 and Advantage monthly. She lived indoors with four cats and there had been no history of swimming.

**Physical exam:** On presentation, Sabrina was bright alert and responsive and weighed 49 lbs. Her mucous membranes were pink with a CRT<2. Conformation, nutritional status and character of coat were within normal limits. Her pulse rate was 84 and strong. Respiratory rate and rectal temperature were not taken at this time. There was a 6" round necrotizing ulcerated full thickness lesion on the base of the tail head. There was eschar over the central part of the lesion. The edge of the lesion was black to dark pink and raised with a visible line of demarcation between the lesion and normal skin.

**Differential diagnoses** included deep pyoderma (*Pseudomonas*, *Staphylococcus*, anaerobic), fungal infection, pythiosis, and mycobacterial infection.

**Plan:** biopsy for histopathology, do cytology and culture the lesion.

## **Histopathology results (Thanks to Dr. William Castleman):**

Sections include haired skin and panniculus. The epidermis within these sections is moderately acanthotic. The superficial dermis is edematous. Throughout the dermis and panniculus are angiocentric collections of neutrophils, macrophages and eosinophils. Occasional giant cells are present. The center of these inflammatory foci are necrotic. Within the superficial dermis, these necropurulent foci involve follicles and adnexa. Dense neutrophil accumulations are within muscular arteries, arterioles and veins. Associated with and surrounding vessels are large numbers of nonseptate fungal hyphae with thick irregular walls and irregular branching. The hyphae are not detectable in H&E sections.

**Final anatomic diagnosis:** severe, chronic, necrotizing and suppurative dermatitis and panniculitis with arteritis and fungal hyphae.

**Comment:** The lesions and staining pattern are consistent with pythiosis although other zygomycete infection can not be completely excluded.

## **DIAGNOSIS**

## **Test Your Knowledge - Diagnosis**

Comment: The lesions and staining pattern are consistent with pythiosis although other zygomycete infection can not be completely excluded.

Diagnosis: probable pythiosis

Final outcome: Sabrina was euthanized due to the poor prognosis that pythiosis has.

Pythiosis is very invasive disease usually found in the Southeast, especially those areas surrounding the Gulf of Mexico. The inciting organism, *Pythium insidiosum*, is often found associated with aquatic environments and it can cause cutaneous, as well as, gastrointestinal pyogranulomatous lesions. Hair from German Shepherd dogs and horses have been found to be predisposed to attracting the zoospores. The classic signalment is a young, male, large-breed dog (the same type of dog that would most likely go and swim in any lake if he had the chance). While *Pythium insidiosum* is not a true fungus, it is a true pathogen and there is a low risk of zoonosis. Unfortunately, pythiosis carries a poor prognosis. Wide surgical excision is the only treatment and the lesions generally recur. Usually the lesions are already diffuse by the time the clinical signs are apparent.

For more information on pythiosis, see The Compendium from January 1998, volume 20, number 1 written by Randall C. Thomas, DVM and Diane T. Lewis, DVM.

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